PHYLOGENETIC ANALYSIS OF BIGNONIACEAE BASED ON THE cpDNA GENE SEQUENCES rbcL AND ndhF[1]

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ABSTRACT

A phylogenetic analysis of the Bignoniaceae and related families was conducted using the DNA sequences of the chloroplast genes rbcL and ndhF. Trees were constructed using each gene separately and in a combined data set. The analysis suggested that the family is more derived within the order Lamiales sensu lato than once believed. Paulownia and Schlegelia previously have been placed in Bignoniaceae or Scrophulariaceae. However, the sequence data presented here do not support their placement in Bignoniaceae. Excluding Paulownia and Schlegelia, Bignoniaceae were found to be monophyletic. Tribes Bignonieae, Crescentieae, and Coleeae each forms a monophyletic group based on this analysis. Tribe Tecomeae is paraphyletic.

Bignoniaceae are called "one of the most important families of woody plants and the most important family of lianas in the Central American forest ecosystems" (Gentry, 1974: 728). Gentry spent much of his career working on this family, and his contributions to Bignoniaceae systematics were extensive. Bignoniaceae are a family of 7 or 8 tribes, 112 genera, and 800 species, composed primarily of lianas, trees, and some shrubs. The family is essentially pantropical, although a few species reach the temperate zone, with 78% (620) of the species occurring in the Neotropics and a center of diversity in Brazil (Gentry, 1980). The origin of the family is not clear; however, Goldblatt and Gentry (1979) speculated that some Old World species may be among the most primitive members of the family. Bignoniaceae are united phylogenetically by such characters as the presence of two distinct placental ridges, each bearing one to several rows of ovules, and lack of endosperm in the mature seeds (Armstrong, 1985). Additionally, a cup-shaped calyx, tubular corolla morphology, woody habit, a chromosome number of $n = 20$, bilamellate stigma with an elongate style, and seeds with a hyaline wing surrounding the embryo are also characteristic taxonomically (Gentry, 1980). However, many members of the Scrophulariaceae share some of these character states, leading to confusion in the placement of morphologically intermediate taxa.

A traditional placement for Bignoniaceae is in the order Scrophulariales (sensu Cronquist, 1981) in subclass Asteridae (Cronquist, 1981; Takhtajan, 1980), considered an evolutionarily derived subclass within the dicots. Molecular studies (Olmstead et al., 1992; Olmstead et al., 1993a) have suggested that the Lamiales (sensu Takhtajan, 1980; primarily Lamiaceae and Verbenaceae) and the Scrophulariales together form a monophyletic group (along with Cronquist's small orders Plantaginales and Callitrichales), but that neither is monophyletic. This expanded group, which we refer to as Lamiales sensu lato, is recognized by Thorne (1992), who nevertheless retained the two traditional groups at the level of suborder within the order. In at least one study based on rbcL (Olmstead et al., 1993a), Bignoniaceae appeared basal in the Lamiales s.l., a placement consistent with traditional expectations (e.g., Goldblatt & Gentry, 1979). However, subsequent molecular studies including more taxa in the Lamiales s.l. and/or additional sequence data (Olmstead & Reeves, 1995; Wagstaff & Olmstead, 1997) found Bignoniaceae nested within the order. Ambiguity arises because characters traditionally used to infer ancestral lineages

in the Lamiales s.l. (e.g., woodiness and many ovules per carpel) co-occur with characters that have been considered characteristics of more recent lineages (e.g., lack of endosperm, climbing habit, winged seeds, and compound leaves).

Early treatments of Bignoniaceae relied on fruit morphology to divide the family into tribes. De Candolle (1838) recognized two tribes: Bignonieae, with dehiscent fruits, and Crescentieae, with indehiscent fruits. Bentham and Hooker (1876) followed his lead but divided de Candolle's tribe Bignonieae into three separate tribes based on locule number and orientation of the septum relative to the capsule valves: Bignonieae, with two locules and the septum parallel to the valves; Tecomeae, with two locules and septum perpendicular to the valves; and Jacarandeae, with one locule and partially or completely missing the septum. Bignonieae and Tecomeae were subtribes in de Candolle's (1838) system, while Jacarandeae was considered an unnatural group. Schumann (1894) distributed the genera of Bentham and Hooker's Jacarandeae among the three large tribes Bignonieae, Crescentieae, and Tecomeae, and recognized two monogeneric tribes, Eccremocarpeae (first described by Endlicher in 1839) and Tourrettieae (first described by Don in 1838). Bojer (1837) created the tribe Coleeae for a small group of Madagascan genera with fleshy, indehiscent fruits. In all subsequent major treatments, these genera usually were included in Crescentieae, until tribe Coleeae was resurrected by Gentry (1976). Tribe Oroxyleae was defined (Gentry, 1980) to include four Old World genera formerly put in Bignonieae that have reduced chromosome numbers ($n = 14, 15$): *Oroxylum*, *Millingtonia*, *Nyctocalos*, and *Hieris* (Gentry, 1980). Chromosome numbers of $n = 20$ are typical in the family and are found almost universally among tribes Bignonieae, Coleeae, Crescentieae, Schlegelieae, and Tourrettieae (Goldblatt & Gentry, 1979). Tribe Schlegelieae was erected to include *Schlegelia*, *Gibsoniothamnus*, *Synapsis*, and recently, *Exarata* (Gentry, 1980, 1992a). Reveal (1995) later segregated tribe Schlegelieae into its own family, Schlegeliaceae, based on the molecular evidence of Olmstead and Reeves (1995). A summary of classifications, key characteristics, and geographic distribution of tribes in Bignoniaceae is presented in Table 1. For a comprehensive discussion of the taxonomic and nomenclatural history of Bignoniaceae, see Gentry (1980).

Gentry (1980) maintained that it is very difficult to understand the phylogeny within Bignoniaceae because of "rampant parallelisms and convergence in nearly every taxonomically important character-

istic." He speculated that tribes Tecomeae and Oroxyleae were "closest to the ancestral stock of Bignoniaceae," although he acknowledged that tribe Schlegelieae may have diverged from the common ancestor that gave rise to both Bignoniaceae and Scrophulariaceae. Oroxyleae were suggested to be primitive on the basis of the presence of five stamens in some members and the putatively ancestral chromosome base number of $x = 14$. Tecomeae were suggested to be primitive on the basis of having a world-wide distribution and a few Old World genera with five anthers. Tecomeae were believed to have given rise to the other tribes with more restricted distributions such as Bignonieae, Coleeae, and Crescentieae (Gentry, 1980). This scenario depicts Tecomeae as a paraphyletic group, and implies that dehiscent fruits with the septum perpendicular to the valves gave rise to indehiscent fruits (Coleeae, Crescentieae) and to dehiscent fruits with the septum parallel to the valves (Bignonieae).

Paulownia and the tribe *Schlegelieae* have both been considered intermediate between Bignoniaceae and Scrophulariaceae, and their placement is still in doubt (Armstrong, 1985; Gentry, 1980). *Schlegelia* and *Paulownia* both have a haploid complement of $n = 20$, suggesting a relationship with the Bignoniaceae (Gentry, 1980; Goldblatt & Gentry, 1979; Westfall, 1949). Presence of endosperm, gynoecial anatomy, embryo and placental morphology, and winged seeds morphologically different from those of Bignoniaceae suggests a relationship with the Scrophulariaceae (Armstrong, 1985). Molecular systematic studies in the Scrophulariaceae (Olmstead & Reeves, 1995) indicated that these taxa may not belong to either family, but to two of many small, independent lineages in the Lamiales s.l. (Wagstaff & Olmstead, 1997).

In the present study, we used sequences from the chloroplast genes *rbc*L and *ndh*F to construct a phylogenetic hypothesis for Bignoniaceae. Recent publications have used these two chloroplast genes for phylogenetic studies within the Asteridae (Olmstead & Sweere, 1994), and even within the Lamiales s.l. (Olmstead & Reeves, 1995; Scotland et al., 1995). The phylogenetic hypothesis based on these gene sequences is here used to determine whether Bignoniaceae are a monophyletic family, especially regarding the phylogenetic relatedness of *Schlegelia* and *Paulownia* to the "core" Bignoniaceae. The position of the family within the Lamiales s.l. is examined. We also discuss the relationships among primary lineages within the Bignoniaceae. The phylogenetic hypotheses complement recent monographic work (D'Arcy, 1997; Gentry, 1980,

Table 1. Tribes recognized in major classifications of Bignoniaceae and features of taxa placed into each tribe. Geographic distribution is listed for tribes recognized by the most recent classification of Gentry (1980).

de Candolle (1838)	Bentham and Hooker (1876)	Schumann (1894)	Gentry (1980)	Geographic distribution of taxa
Crescentieae	Crescentieae	Crescentieae	Crescentieae	Central America and the West Indies
Indehiscent fruit	Indehiscent fruit	Indehiscent fruit and seeds without wings	Trees with indehiscent fruits; seed wings can be vestigial, New World distribution	
Bignonieae	Bignonieae	Bignonieae	Bignonieae	New World tropics
Dehiscent fruit	Two locules in the fruit; septum parallel to the valves	Two locules in the fruit; septum parallel to the valves	Mostly lianas with two locules in the fruit; septum parallel to the valves	
	Tecomeae	Tecomeae	Tecomeae	Pantropical
	Two locules in the fruit; septum perpendicular to the valves	Two locules in the fruit; septum perpendicular to the valves	Trees with two-loculate fruit and the septum perpendicular to valves	
	Jacarandeae	Eccremocarpeae	Eccremocarpeae	Andes
	Unilocular fruit with a partial or missing septum	Unilocular fruit opening downward; spiny and apically fused capsule	Unilocular fruit opening downward; spiny and apically fused capsule	
		Tourrettieae	Tourrettieae	Andes
		Four-locular fruit	Four-locular fruit	
			Coleeae	Madagascar and tropical Africa
			Indehiscent fruits with an Old World distribution	
			Schlegelieae	Central and South America and Cuba
			Lianas with indehiscent fruits and seed wings developed, but see *Exarata* (Gentry, 1992a)	
			Oroxyleae	Southeast Asian tropics
			Chromosome numbers of $n = 14$ or 15, not $n = 20$ as in most of the rest of the family	

1992b; Hauk, 1997), as well as continuing systematic studies on the family (M. Zjhra, pers. comm.; G. Dos Santos, pers. comm.). The monographic studies deal primarily with taxa from the New World, whereas this study provides a framework phylogeny for the entire family, including both Old and New World taxa.

MATERIALS AND METHODS

This study included sequences from 19 species of Bignoniaceae plus *Paulownia* and *Schlegelia*, an additional 14 members of the Lamiales s.l., and 4 outgroup taxa from related orders (Table 2). Seven tribes of Bignoniaceae are represented (following Gentry, 1980), in addition to *Paulownia* (sometimes included in the tribe Tecomeae) and *Schlegelia* (Schlegeliaceae, or formerly in Bignoniaceae tribe Schlegelieae). Only the monotypic tribe Tourrettieae was not included in this study because DNA was not readily available at the time of this study.

Total genomic DNA was extracted from leaf material using the CTAB procedure (Doyle & Doyle, 1987), followed by cesium-chloride ultracentrifugation. The chloroplast genes *rbcL* and *ndhF* were amplified following a two-stage PCR process to generate single-stranded DNA (Kaltenboeck et al., 1992). Sequencing was done using ³²P-labeled dideoxy nucleotides, and sequences were visualized using autoradiography. Amplification and internal primer sequences for each gene and details of sequencing methods were as in Olmstead et al. (1992, 1993a, b), Olmstead and Sweere (1994), and Olmstead and Reeves (1995). Both DNA strands were sequenced and compared for all taxa to ensure accuracy, and sequences generated for the present study were submitted to GenBank. Both *rbcL* and *ndhF* sequences for each taxon were obtained from the same accession except for *Nicotiana, Barleria, Digitalis,* and *Verbena*. Sequences were aligned manually, and "gaps" introduced into sequences due to taxon specific insertion/deletion events were not treated as separate characters. Missing data were coded as a question mark in the data matrix.

The sequences were analyzed using a test version of PAUP* version 4.0d56 (kindly provided by D. Swofford) on a Sun Ultra 1 computer. The *rbcL* and *ndhF* data sets each were analyzed separately and in a combined data set. For each data set (*rbcL, ndhF,* and combined) a heuristic search of 100 replications with random order taxon entry, TBR branch swapping, and MULPARS was used to find the shortest trees. Bootstrap analyses (Felsenstein, 1985) were conducted as a quantitative evaluation of the relative strength of monophyletic groupings

in each tree. The *ndhF* and combined data sets had 100 bootstrap replicates with TBR swapping and MULPARS in effect, and the *rbcL* data set underwent 1000 bootstrap replicates with MULPARS off. A decay analysis (Donoghue et al., 1992) was performed on the combined data using the computer program AutoDecay version 2.9.9 (Eriksson, 1997). The data in the combined analysis were partitioned into their respective *rbcL* and *ndhF* components, and an Incongruence Length Difference Test (partition-homogeneity test) (Farris et al., 1995) was conducted to determine if the partitions differed significantly from random partitions of the combined data. A maximum-likelihood analysis using the discrete gamma-approximation model was conducted on the combined data set for comparison with the parsimony analysis. The trees resulting from these two analyses were compared using the Kishino-Hasegawa test (Kishino & Hasegawa, 1985) under the Hasegawa-Kishino-Yano (Hasegawa et al., 1985) likelihood model using a discrete gamma approximation.

RESULTS

Sequence data from the two chloroplast genes included a total of 3545 bp of aligned sequence, with 709 phylogenetically informative sites. The *rbcL* sequences were 1411 bp in length (40% of the total sequence in the combined data set), and had 194 phylogenetically important characters (14% of the total *rbcL* sequence length and 27% of the total number of informative characters in the combined data). Aligned *ndhF* sequences were 2134 bp in length (60% of the total in the combined data set) with 515 phylogenetically informative characters (24% of the total *ndhF* sequence length and 73% of the total number of informative characters in the combined data). One 6 bp insertion was found in all taxa except *Nyctanthes* and the outgroups, and a single shared deletion occurred in *Petunia* and *Nicotiana*. Each of these indels denotes groups with 100% bootstrap support based on nucleotide substitutions. Another insertion was shared between two distantly related taxa, *Radermachera* (Bignoniaceae) and *Nematanthus* (Gesneriaceae), but there is overwhelming evidence from the remainder of the sequence data against their grouping.

The analysis of the *rbcL* sequences generated 63 equally parsimonious trees of 826 steps. These trees occurred in islands of 15 and 48 trees (called tree island-15 and tree island-48, respectively). One of the most parsimonious trees is depicted with branch lengths in Figure 1, along with the strict

consensus of all 63 trees. Bignoniaceae formed one of several clades emerging from a basal polytomy in the Lamiales s.l. The family formed a monophyletic group in the *rbc*L tree, but with low bootstrap support. *Schlegelia* and *Paulownia* emerged as independent lineages from this polytomy and did not occur in a clade with the Bignoniaceae in any of the 63 trees. Tribe Tecomeae (taxa denoted with a "T" in Fig. 1) was not monophyletic within the family, while the other tribes were monophyletic (Bignonieae, Coleeae, and Crescentieae) or were represented by only one taxon in this analysis (Oroxyleae and Eccremocarpeae).

The *ndh*F analysis yielded four trees of 2420 steps (Fig. 2). Bignoniaceae were not found to be monophyletic, but a subset of Bignoniaceae, excluding *Jacaranda* and *Podranea*, formed a single clade with 82% bootstrap support. Resolution and support for internal nodes below the ordinal level was greater for the *ndh*F tree than for the *rbc*L tree. *Jacaranda* and *Podranea*, both usually included in the family (tribe Tecomeae), formed a weakly supported group with *Schlegelia* separate from the rest of the Bignoniaceae. A search constraining *Jacaranda* and *Podranea* to monophyly with the rest of Bignoniaceae resulted in trees only two steps longer (length = 2422). Constraining *Schlegelia* and these two taxa to a monophyletic Bignoniaceae required one additional step (length = 2423). *Paulownia* formed a well-supported clade with the Lamiaceae that was sister to a weakly supported clade composed of the majority of Bignoniaceae + *Sesamum* (Pedaliaceae).

The parsimony analysis of the combined *rbc*L and *ndh*F sequences produced three most-parsimonious trees of 3285 steps (Fig. 3). The partition-homogeneity test resulted in a value of $P = 0.01$, so the null hypothesis that the partitions represent two random partitions of the same homogeneous data pool was statistically rejected by this test. The combined analysis identified a monophyletic Bignoniaceae, excluding *Paulownia* and *Schlegelia*. Maximum likelihood analysis produced a tree (not shown) almost identical to the combined tree, except in the ML tree *Catalpa* and *Oroxylum* did not form a clade, *Cydista* and *Martinella* switched places within tribe Bignonieae, *Jacaranda* was the sister group to the rest of Bignoniaceae, *Stachytarpheta* was sister to the clade containing *Antirrhinum*, *Digitalis*, *Barleria*, and *Sesamum* (rather than forming a clade with *Verbena*), and a clade comprised of *Schlegelia* and *Verbena* was sister to Bignoniaceae. The tribes within the Bignoniaceae were still monophyletic in the ML tree, with the exception of a paraphyletic Tecomeae, as also

seen in the other analyses. The Kishino-Hasegawa test was not significant ($P = 0.60$), indicating statistical congruence between the ML and the parsimony trees despite minor topological differences. In the parsimony tree, constraining *Schlegelia* with Bignoniaceae added two steps, constraining *Paulownia* with Bignoniaceae added six steps, and constraining *Schlegelia* and *Paulownia* with Bignoniaceae added seven steps.

DISCUSSION

The increased internal resolution for the *ndh*F alone trees relative to the *rbc*L trees is consistent with previous studies (Olmstead & Reeves, 1995; Olmstead & Sweere, 1994; Wagstaff et al., 1998). Phylogenetic signal from *rbc*L becomes more apparent when data sets are combined. The combined data provide a more reliable hypothesis of relationships than either gene tree alone, taking the signal from both genes and producing a better resolved tree with increased statistical support, as also seen by Olmstead and Sweere (1994). For example, Bignoniaceae were found to be monophyletic with *rbc*L, but with only ca. three nucleotide substitutions and 14% bootstrap support, and were not found to be monophyletic with *ndh*F. However, with the combined data, Bignoniaceae have 15 inferred substitutions and bootstrap support of 63%. In general, areas of low support in the combined data tree reflect lack of support in both of the individual gene trees. This is not the case, though, for the *Pandorea* + *Podranea* and the *Tecoma* + *Pandorea* + *Podranea* clades; in the *rbc*L tree they have 85% and 80% bootstrap support, respectively, and they do not occur in the combined tree at all.

The result that the partitions of the data (in this case, each gene sequence) are not random partitions of a homogeneous data pool may reflect dependence of unknown cause for some characters within one or both gene sequences. Congruence tests are sensitive to localized regions of heterogeneity and can produce significant results even when a small portion of a single partition is giving misleading results. Fine partitioning of data sets may help to identify problem areas that would lead to rejection of homogeneous data partitions, but reasons for the incongruence would still not be revealed. However, because the two genes sampled here were drawn from the same non-recombining genome, they are presumed to have the same underlying phylogeny, and the combined data set was assumed to give the best estimate of phylogeny for the group. Other data sets using the same two regions of the genome have yielded similar results for

Table 2. Taxon voucher information and GenBank numbers for sequences used in the analyses. References are listed for voucher information of previously published sequences. Taxa are arranged according to the classification of Thorne (1992).

Taxon	Voucher specimen, deposit location, and references	GenBank number for *rbc*L sequences	GenBank number for *ndh*F sequences
GENTIANALES			
Gentianaceae			
Gentiana procera Holm.	none cited (Olmstead et al., 1993a; Olmstead & Reeves, 1995)	L14398	L36400
BORAGINALES			
Boraginaceae			
Borago officinalis L.	none cited (Olmstead et al., 1992; Olmstead & Reeves, 1995)	L11680	L36393
SOLANALES			
Solanaceae			
Nicotiana tabacum L.	(Lin et al., 1986; Olmstead et al., 1993b)	M16896	L14953
Petunia parviflora Juss.	*A. C. Sanders 5835* (COLO) (Olmstead et al. 1998, in press)	In press	In press
LAMIALES s.l.			
Acanthaceae			
Barleria prionitis L.	*Uppsala B.G. 1977-3036* (UPS) (Chase et al., 1993; Scotland et al., 1995)	L01886	U12653
Bignoniaceae			
Amphitecna apiculata A. H. Gentry	*R. Spangler B1* (MO)	AF102640	AF102624
Arrabidaea pubescens (L.) A. H. Gentry	*A. H. Gentry 10234* (MO)	AF102641	AF102625
Campsis radicans Seem.	DNA from R. Jansen, voucher unknown	AF102642	AF102626
Catalpa sp.	*C. W. dePamphilis s.n.* (COLO) (Olmstead et al., 1992; Olmstead & Reeves, 1995)	L11679	L36397
Crescentia portoricensis Britton	*Gentry & Zardini 50458* (MO)	AF102643	AF102627
Cybistax donnell-smithii (Rose) Seibert	*Collector unknown*, Waimea Bot. Gard. 89p166	AF102644	AF102628
Cydista aequinoctialis Miers	*R. Spangler B2* (MO)	AF102645	AF102629
Eccremocarpus scaber Ruiz & Pav.	*Chase 2999* (K)	AF102646	AF102630
Jacaranda sparrei A. H. Gentry	*H. Descimmon s.n.*, Waimea Bot. Gard. 82s772	AF102647	AF102631
Kigelia africana Benth.	*R. C. A. Rica s.n.*, Waimea Bot. Gard. 74s980	AF102648	AF102632
Macfadyena unguis-cati (L.) A. H. Gentry	*R. Spangler B3* (MO)	AF102649	AF102633
Martinella obovata Bureau & K. Schum.	*A. H. Gentry 50277* (MO) (Olmstead & Reeves, 1995)	L36444	L36402
Ophiocolea floribunda (Boj. ex Lindl.) H. Perrier	*G. Schatz 3448* (MO)	AF102650	AF102634
Oroxylum indicum (L.) Kurz	*A. H. M. Jayasuriya s.n.*, Waimea Bot. Gard. 79s51	AF102651	AF102635
Pandorea jasminoides Schum.	*Collector unknown*, Matthaei Bot. Gard. Ann Arbor, Michigan	AF102652	AF102636

Table 2. Continued.

| Taxon	Voucher specimen, deposit location, and references	GenBank number for rbcL sequences	GenBank number for ndhF sequences
Podranea ricasoliana Sprague	Collector unknown, Waimea Bot. Gard. 84p524	AF102653	AF102637
Radermachera frondosa Chun & How	Gentry & Ortiz 78009 (MO)	AF102654	AF102638
Tabebuia heterophylla (A. deCandolle) Britton	none cited (Olmstead & Reeves, 1995)	L36451	L36416
Tecoma stans Juss.	DNA from R. Jansen, voucher unknown	AF102655	AF102639
Buddlejaceae			
Buddleja davidii Franch.	R. G. Olmstead 88-007 (WTU) (Olmstead et al., 1993a; Olmstead & Reeves, 1995)	L14392	L36394
Gesneriaceae			
Nematanthus hirsutus (Mart) Wiehler	Collector unknown (SEL) (Olmstead & Reeves, 1995)	L36446	L36404
Lamiaceae			
Callicarpa dichotoma (Lour.) K. Koch	R. G. Olmstead 88-012 (WTU) (Olmstead et al., 1993a; Olmstead & Reeves, 1995)	L14393	L36395
Lamium purpureum L.	S. J. Wagstaff 88-031 (BHO) (Wagstaff & Olmstead, 1997; Wagstaff et al., 1988)	U75702	U78694
Myoporaceae			
Myoporum mauritianum A. de Candolle	R. G. Olmstead 92-299 (WTU) (Olmstead & Reeves, 1995)	L36445	L36403
Oleaceae			
Nyctanthes arbor-tristis L.	RBG, Kew 099.86.00993 (K) (Wagstaff & Olmstead, 1997)	U28877	U78708
Pedaliaceae			
Sesamum indicum L.	none cited (Olmstead et al., 1993a; Olmstead & Reeves, 1995)	L14408	L36413
Scrophulariaceae			
Antirrhinum majus L.	C. W. dePamphilis s.n. (Olmstead et al., 1992; Olmstead & Reeves, 1995)	L11688	L36413
Digitalis grandiflora Lam.	none cited (Olmstead & Reeves, 1995)		L36399
Digitalis purpurea L.	none cited (Olmstead et al., 1993a)	L01902	
Paulownia tomentosa (Thunb.) Steudel	R. G. Olmstead 88-008 (WTU) (Olmstead & Reeves, 1995)	L36447	L36406
Schlegelia parviflora (Oerst.) Monachino	Gentry & Puig-Ross 14221 (MO) (Olmstead & Reeves, 1995)	L36448	L36410
Scrophularia sp.	C. W. dePamphilis s.n. (Olmstead & Reeves, 1995)	L36449	L36411
Verbascum thapsus L.	none cited (Olmstead & Reeves, 1995)	L36452	L36417
Verbenaceae			
Stachytarpheta dichotoma (Ruiz et. Pav.) Vahl.	R. G. Olmstead 951 (WTU) (Olmstead & Reeves, 1995)	U32161	L36414
Verbena bonariensis L.	none cited (Olmstead et al., 1993a)	L14412	
Verbena bracteata Lagasca & Rodriguez	R. G. Olmstead 92-131 (WTU) (Olmstead & Reeves, 1995)		L36418
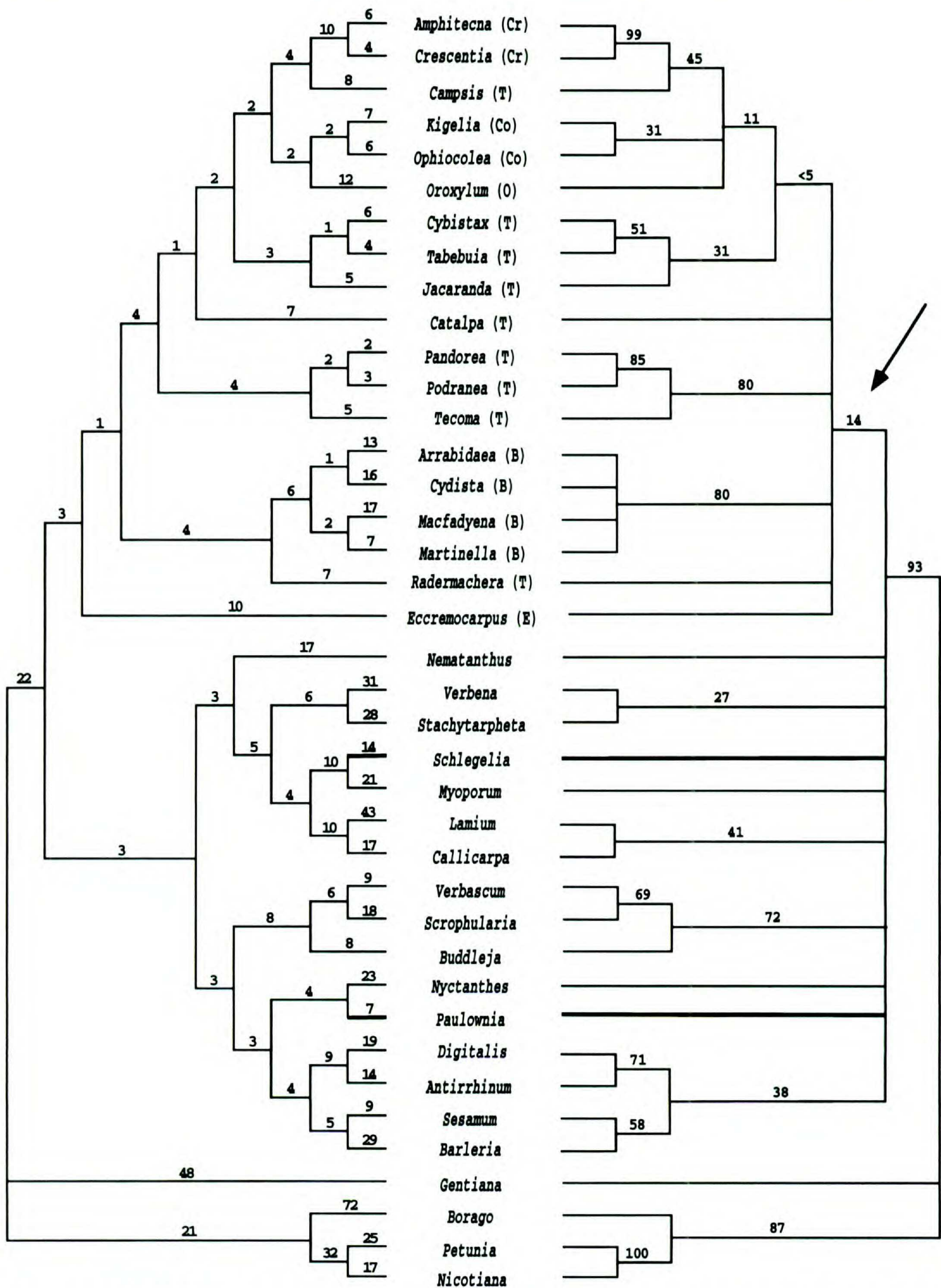
Figure 1. On the left is one of the most parsimonious trees from *rbc*L tree island-48 (length = 826, CI = 0.58, RI = 0.48). Branch lengths are indicated. On the right is the strict consensus of all 63 trees found with bootstrap values indicated. The arrow indicates the internode defining the Bignoniaceae. A letter next to a taxon name designates the tribe that taxon is a member of (B = Bignonieae, Co = Coleeae, Cr = Crescentieae, E = Eccremocarpeae, O = Oroxyleae, T = Tecomeae). Bold branches indicate *Schlegelia* and *Paulownia*.
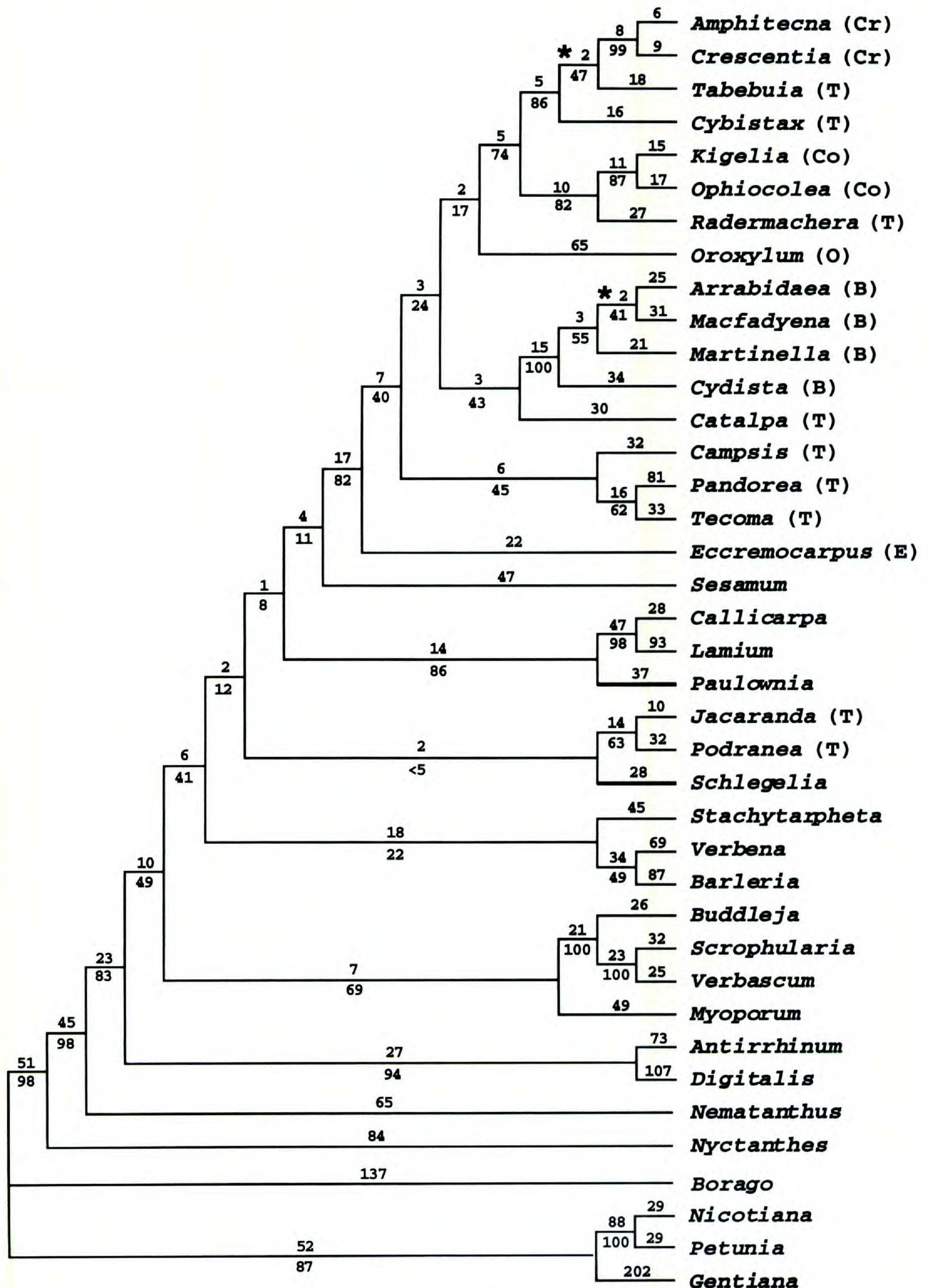
Figure 2. One of four most parsimonious trees based on the *ndh*F sequences. Branch lengths are indicated above branches, bootstrap numbers are indicated below. Asterisks indicate internodes that collapse in the strict consensus of all four trees (length = 2432, CI = 0.56, RI = 0.48). A letter next to a taxon name designates the tribe that taxon is a member of (B = Bignonieae, Co = Coleeae, Cr = Crescentieae, E = Eccremocarpeae, O = Oroxyleae, T = Tecomeae). Bold branches indicate *Schlegelia* and *Paulownia*.
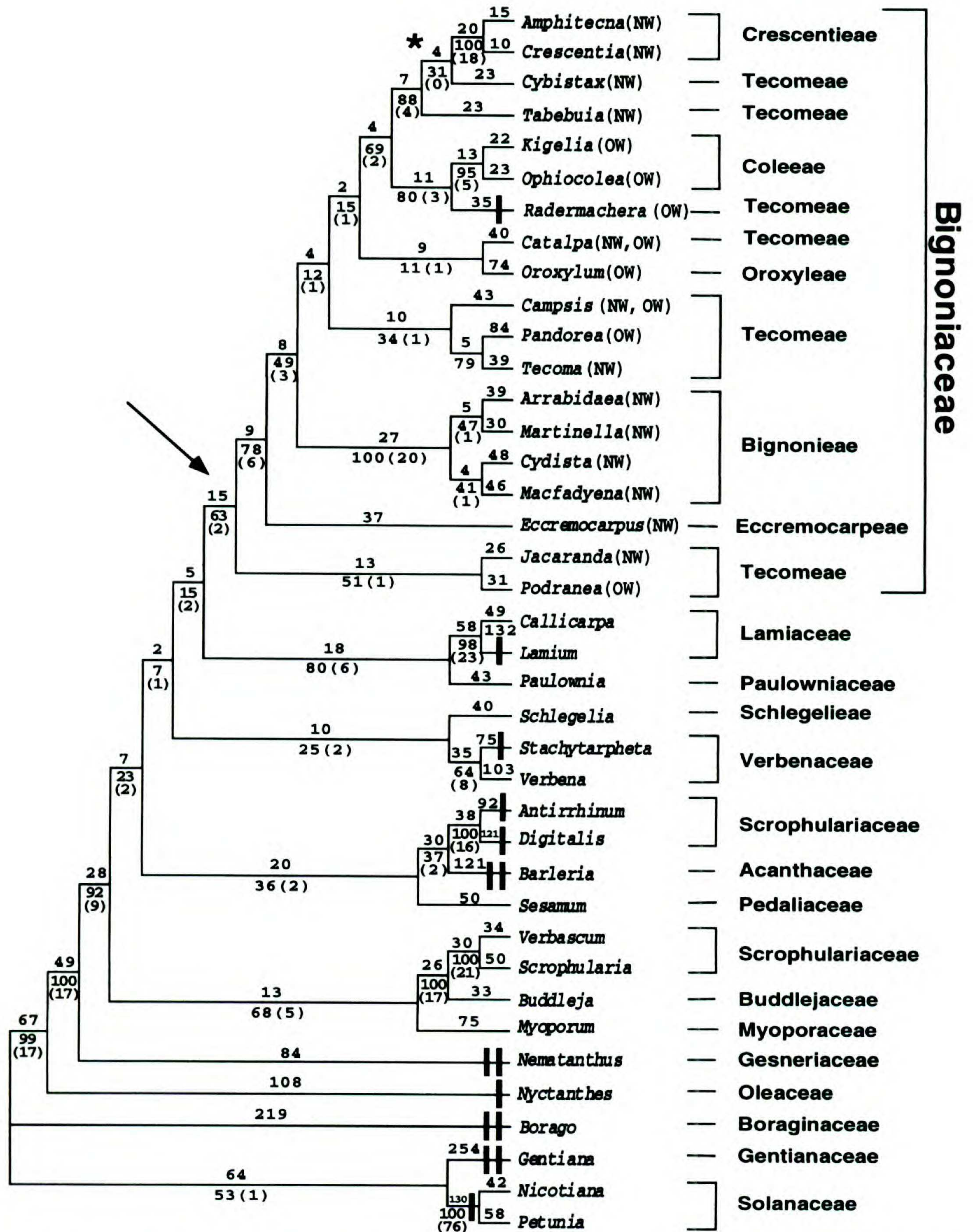
Figure 3. One of three most parsimonious trees based on the combined *rbc*L and *ndh*F data (length = 3285, CI = 0.56, RI = 0.47). Branch lengths are indicated above branches. Bootstrap numbers are below internodes with decay values in parentheses. The arrow denotes the clade comprising the Bignoniaceae. "NW" indicates a New World member of the Bignoniaceae, "OW" designates the taxon occurs in the Old World. The asterisk denotes the only internode that collapses in the strict consensus of the three trees, and vertical bars indicate inferred insertions or deletions.

this test of congruence. Data from Solanaceae (Olmstead & Sweere, 1994) gave a value of $P = 0.04$, and data from Scrophulariaceae (Olmstead & Reeves, 1995) had $P = 0.01$, significant in both cases. Lamiaceae (Wagstaff et al., 1998), on the other hand, gave $P = 0.39$ for the partition-homogeneity test, a non-significant result. Beyond the inherent problems congruence tests have, it remains unclear why these independent data sets, each representing groups of relatively closely related species, gave results indicating significant heterogeneity between data sets for these genes.

Bignoniaceae monophyly based on the combined cpDNA data agrees with the hypothesis Gentry (1980) proposed based on morphological characters, except for *Schlegelia*. Gentry considered *Schlegelia* to be part of, but distant from, the rest of Bignoniaceae (see discussion below). Morphological characters shared between Bignoniaceae and Scrophulariaceae that have caused confusion in the placement of taxa such as *Paulownia* and *Schlegelia* can now be examined in the context of the molecular data. True morphological synapomorphies for Bignoniaceae may be characters such as lack of endosperm, compound leaves, and unique type of winged seeds. A clear sister group to Bignoniaceae based on the present results is not evident. The *rbc*L analysis leaves relationships unresolved among the Lamiales s.l., the *ndh*F analysis places Pedaliaceae as sister to Bignoniaceae, and the combined data analysis places *Paulownia* sister to Lamiaceae, all with low support. Indeed, there is no evidence that Scrophulariaceae, or any part of this family (Olmstead & Reeves, 1995), are sister to Bignoniaceae. Future studies using morphology, cytology, and anatomy (as formerly done by Armstrong, 1985; Guédés, 1974; Westfall, 1949) to decipher the connection between these groups can now make use of a more complete phylogenetic hypothesis to direct the scope of their comparisons.

The sequence data do not support Bignoniaceae as a basal lineage in the Lamiales s.l., as suggested by Goldblatt and Gentry (1979); rather, they are nested high in the order. Oleaceae (*Nyctanthes*) are sister to the rest of the order in the combined analysis. This result agrees with other previously published studies (Hedren et al., 1994; Olmstead et al., 1993a; Wagstaff & Olmstead, 1997). Woody habit has been used as a character indicating a basal placement of Bignoniaceae in the Lamiales s.l., but it cannot be polarized easily due to its widespread occurrence in the order and poor resolution among clades.

Clues to the placement of the problematic genera *Paulownia* and *Schlegelia* can be found in the re-

sults presented here (Figs. 1–3). However, limited sampling among outgroup families, particularly the polyphyletic Scrophulariaceae, limits the strength of any conclusions concerning their placement. Various molecular studies (Olmstead & Reeves, 1995; Wolfe et al., 1997; Nickrent et al., 1998; Wolfe & dePamphilis, 1998; Young et al., 1997) have all focused on particular groups of Lamiales s.l., and sampling has not been comprehensive in any one study with respect to all possible placements for these two taxa. Several studies in press have included *Paulownia* and/or *Schlegelia* in surveys of the chloroplast genes *rbc*L, *ndh*F, *mat*K, and *rps*2. A large study using three genes and many taxa from the Scrophulariaceae (Olmstead et al., unpublished) suggests that *Paulownia* belongs with the Orobanchaceae (broadly defined to include all parasitic Scrophulariaceae), but weakly so (no members of Orobanchaceae were included in the present study or in Olmstead & Reeves, 1995). The results of the unpublished study of Olmstead et al. grouped *Schlegelia* with Bignoniaceae, but with weak support, and only *Kigelia* and *Catalpa* were included as Bignoniaceae representatives. The results here and in other molecular studies (Olmstead & Reeves, 1995; Wolfe et al., 1997; Nickrent et al., 1998; Wolfe & dePamphilis, 1998; Young et al., 1997; Olmstead et al., unpublished) contradict Westfall's (1949) inference, based on cytology, that *Paulownia* belongs in Bignoniaceae, whereas some studies also contradict Armstrong's (1985) placement of *Paulownia* with the Scrophulariaceae (Olmstead & Reeves, 1995; Wolfe et al., 1997). Still other studies suggest a connection to the Orobanchaceae s.l. (Nickrent et al., 1998; Wolfe & dePamphilis, 1998; Young et al., 1997; Olmstead et al., unpublished data) or Lamiaceae (this study). *Schlegelia* has not been found to belong with any group of the Scrophulariaceae in any of the molecular studies in which it has been included. However, some studies suggest *Schlegelia* may be related to Bignoniaceae (Nickrent et al., 1998, *rps*2 sequences; Young et al., 1997; Olmstead et al., unpublished data), while others contradict that relationship (Olmstead & Reeves, 1995; Nickrent et al., 1998, *rbc*L sequences; Wolfe & dePamphilis, 1998). Perhaps the only safe conclusions are that *Paulownia* does not belong with Bignoniaceae and that *Schlegelia* does not belong with Scrophulariaceae.

Restricting Bignoniaceae to the moderately supported clade that excludes tribe Schlegelieae and *Paulownia* better reflects our current uncertainty about the true placement of these problematic taxa. If, one day, one of these groups is found to be sister

to the core Bignoniaceae, a classification that considers them as separate groups still will be consistent with phylogeny. We can now identify the morphological characters that have led to confusion in placement of these taxa, and the actual synapomorphies for Bignoniaceae may be characters such as the lack of endosperm, compound leaves, and unique type of winged seeds. It may be best for us now to recognize Schlegelieae and *Paulownia* as distinct families, rather than fit them into existing families. Nakai (1949) recognized the family Paulowniaceae, separating *Paulownia* from the Bignoniaceae based on the presence of endosperm in the seeds. The family name Schlegeliaceae has been proposed (Reveal, 1995) to include the four genera comprising the tribe Schlegelieae. Reveal's proposals for the recognition of many of the lineages in the Lamiales s.l. are a step forward in bringing the taxonomy of the order into agreement with phylogeny. However, his recognition of the Crescentiaceae (after Dumortier, 1829) would render the Bignoniaceae paraphyletic (see below). Caution should be used in making family-level classifications at this time, when our knowledge of relationships within the Lamiales is still incomplete. Current work on a molecular phylogeny of the entire Lamiales s.l. (R. Olmstead, unpublished data) should provide a framework for a new family-level classification upon its completion.

Within Bignoniaceae, clades corresponding to the tribes Crescentieae, Bignonieae, and Coleeae are well supported. These clades are in agreement with circumscriptions suggested by previous authors (Gentry, 1980; Goldblatt & Gentry, 1979). Tecomeae, however, do not form a monophyletic group in any of the trees presented here, and the Crescentieae, Bignonieae, Coleeae, Oroxyleae, and Eccremocarpeae are each derived from within Tecomeae.

Gentry (1980) considered the indehiscent fruits of the Crescentieae to be derived from the dehiscent fruits of the neotropical Tecomeae. The derivation of Crescentieae from within the Tecomeae is strongly supported in all analyses done for the present study. *Tabebuia* groups strongly with tribe Crescentieae (88% bootstrap in Fig. 3), and in the absence of a name to recognize this strongly supported clade, expanding Crescentieae to include *Tabebuia* may warrant consideration. Our data support recognition of Crescentieae as a distinct taxon within Bignoniaceae; however, Reveal's (1995) recognition of a family Crescentiaceae would make the rest of Bignoniaceae paraphyletic.

Bignonieae are the largest tribe in the family, with their center of diversity in Brazil. All trees

from this study show Bignonieae to be monophyletic, consistent with Gentry's (1980) hypothesis. However, our limited sampling makes this conclusion preliminary. Additional sampling from this group would enhance our knowledge of lineages that compose the family. Gentry (1976, 1980) segregated tribe Oroxyleae from the rest of Bignonieae and suggested it is allied with tribe Tecomeae. Our results confirm this by placing it among the lineages that together comprise the paraphyletic tribe Tecomeae, sister to *Catalpa*. The unique chromosome numbers of Oroxyleae ($n = 14, 15$) indicate a possible synapomorphy for this tribe. Gentry and Tomb (1979) believed the base chromosome number of Bignoniaceae to be $x = 7$, and on this basis, plus the retention of five stamens, placed Oroxyleae basal within the family, or even within the Tubiflorae (essentially Lamiales s.l.) as a whole. These hypotheses have no support from the molecular data. *Jacaranda*, one of the basalmost Bignoniaceae, has four stamens and one staminode, and a chromosome number of $n = 18$. A chromosome count has not been published for *Podranea*.

Our analysis places the monogeneric Andean tribe Eccremocarpeae near the base of Bignoniaceae. Three species were recognized in a recent revision of the tribe (D'Arcy, 1997), but previous treatments recognized as many as six species (Gentry, 1980; Gentry & Tomb, 1979). The clade comprising *Eccremocarpus* and all Bignoniaceae except *Jacaranda* and *Podranea* is strongly supported (78% bootstrap), yet has not been formally named. We declined to name this clade at this point due to limited sampling. Further sampling from Tecomeae is necessary to more rigorously evaluate the monophyly of the Eccremocarpeae.

The paraphyly of tribe Tecomeae presents some major taxonomic problems. The basalmost Bignoniaceae (*Jacaranda* and *Podranea* in Fig. 3) belong to Tecomeae, but other members of this tribe (e.g., *Cybistax*, *Tabebuia*, *Radermachera*) are highly derived within the family. Gentry (1974) suggested that Tecomeae was phylogenetically basal relative to Bignonieae. However, the evidence presented here suggests Bignonieae as sister to much of the rest of the family, including most of the Tecomeae, although support for several branches near the divergence of Bignonieae is weak. Most Tecomeae, as currently circumscribed, share features such as fruits dehiscing perpendicular to the septum and an arborescent habit, but these may be plesiomorphies. Members with palmately compound leaves are usually found in the Neotropics ("NW" in Fig. 3), and the Old World members ("OW" in Fig. 3) usually have pinnately compound leaves. Gentry

(1980) used this information to suggest that Old and New World taxa may represent distinct groups derived from different ancestors within the tribe. The molecular evidence presented here does not support a simple split along geographic lines. It is the only tribe occurring in both the Old and New Worlds, but given the paraphyly of the tribe (Fig. 3), biogeographic interpretations within the tribe have little meaning. The tribe should be split into several smaller lineages (R. Olmstead, unpublished data), or placed with existing lineages where data strongly support groupings. For example, *Radermachera* and tribe Coleeae form a clade with strong support (80% bootstrap in Fig. 3), so these data suggest expanding tribal limits of Coleeae to include *Radermachera*. Taxonomic revision of tribal boundaries in Bignoniaceae should focus on redefining Tecomeae to identify monophyletic lineages.

Increased sampling among members of Bignoniaceae has provided further information for the delimitation of the family. Additional support is provided for the exclusion of *Paulownia* and *Schlegelia* as well as for tribal relationships traditionally based on morphological reproductive characters. It is hoped that the present work will continue to expand our knowledge of Bignoniaceae by providing a phylogenetic framework for current and future work on the family.

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